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PROPHYLACTIC METHODS IN PREVENTION OF DISEASE AMONG ARMY PERSONNEL.

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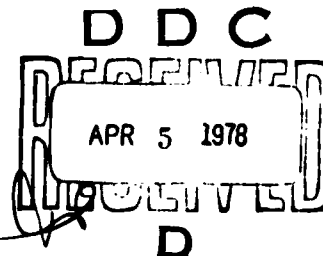
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by

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Surveillance studies to determine the etiologic agents of acute respiratory dis- ease (ARD) in basic combat trainees (BCT's) were accomplished during FY 77. > These studies included 6,897 BCT's hospitalized with ARD at 8 forts, including Ft Ord the only non-BCT fort, plus the U.S. Naval Training Center in San Diego, CA. A special influenza surveillance program was also conducted at Forts Wood, Jackson and Bliss, to signal an early warning of a probable A/Swine flu epidemic. Virus isolations and Serological Studies indicated that 19.9% of ARD hospital- izations were caused by Adenoviruses, 4.5% by Influenza A & B, Mycoplasma, → next page		

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20. Abstract (Contd)

Coxsackie A 21 and Polio; and 74.7% by agents that were not determined.) Of the Adenovirus isolates (1,073), 87.7% (941) were Adenovirus type 21, 8% (86) type 7, 3.6% (39) type 4, 0.37% (4) type 3, and 0.28% (3) type 2. Laboratory titrations of virus in the vaccine pills indicated acceptable potency, $10^{6.0}$ TCID₅₀ for adeno 7, and $10^{5.0}$ for adeno 4. Adeno 21 vaccine was not administered during the year. Two Influenza vaccines were administered during the year - a bivalent vaccine containing 400 CCA Units each of A/Vic/3/75 (H₃N₂) and A/NJ/8-9/76 (H₁N₁); and a monovalent vaccine containing 500 CCA Units of B/HK/5/72. Influenza hospitalizations totaled 448; 61% (272) Flu A, and 39% (176) Flu B infections. Two parainfluenza type 3 infections were identified at Ft. Bliss. In individuals who were hospitalized and seroconverted before 2 wks. post-vaccine, it could not be adequately differentiated between vaccine and infection antibody in the absence of a virus isolation. Thirty-five mycoplasma infections were identified.

Field studies to determine the immunogenicity of Adenovirus Vaccines were conducted utilizing 360 serum pairs collected from 6 Training Forts. Accumulative results indicated the type 4 vaccine to be 69% immunogenic, and the type 7 was 63.6% immunogenic.

A preliminary study to determine the effectiveness of Charcoal Viral Transport Media (CVTM), and bentonite media for transporting virus specimens was conducted using Tryptose Phosphate Broth (TPB) as a control. The recovery of Vaccinia, ECHO 9, Coxsackievirus, Polio, Adenovirus, Herpes, Mumps, and Influenza were similar, but neither media maintained virus titers as adequately as TPB.

Studies to determine whether Coxsackievirus is a significant cause of upper-respiratory infections revealed that this agent represented 25%, 36/144, of the total number of viruses isolated during the period July thru December. Sero-logical data confirms Coxsackievirus A21 as a significant cause of ARD.

During an 8 month period of FY 77, 77 male and female military patients were screened for penicillinase producing *N. Gonorrhoeae* (PPNG). These individuals were selected from Far East arrivals and local area treatment failures. One PPNG was isolated; 3 other penicillin resistant gonococci were isolated, however, they were beta-lactimase negative.

A total of 115 *E. Coli* that were cultured from 23 acute gastroenteritis infants were serotyped and tested for enterotoxin production. The purpose of this study was to correlate the occurrence of sero-pathogenic *E. coli* in gastroenteritis infants, with the presence of enterotoxin. Isolates totaling 137 and 98 from other clinical and non-clinical specimens respectively showed 37 and 26 enteropathogenic *E. coli* respectively, but no enterotoxin was identified.

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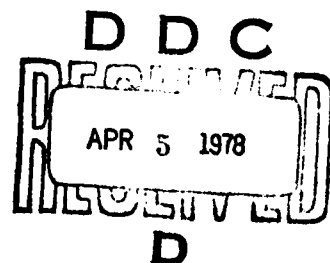
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ABSTRACT

Surveillance studies to determine the etiologic agents of acute respiratory disease (ARD) in basic combat trainees (BCT's) were accomplished during FY 77. These studies included 6,897 BCT's hospitalized with ARD at 8 forts, including Ft. Ord the only non-BCT fort, plus the U.S. Naval Training Center in San Diego, CA. A special influenza surveillance program was also conducted at Forts Wood, Jackson and Bliss, to signal an early warning of a probable A/Swine flu epidemic. Virus isolations and Serological Studies indicated that 19.9% of ARD hospitalizations were caused by Adenoviruses, 4.5% by Influenza A & B, Mycoplasma, Coxsackie A21 and Polio; and 74.7% by agents that were not determined. Of the Adenovirus isolates (1,073), 87.7% (941) were Adenovirus type 21, 8% (86) type 7, 31.6% (39) type 4, 0.37% (4) type 3, and 0.28% (3) type 2. Laboratory titrations of virus in the vaccine pills indicated acceptable potency, $10^{6.0}$ TCID₅₀ for adeno 7, and $10^{5.0}$ for adeno 4. Adeno 21 vaccine was not administered during the year. Two Influenza vaccines were administered during the year - a bivalent vaccine containing 400 CCA Units each of A/Vic/3/75 (H₃N₂) and A/NJ/8-9/76 (H₁N₁); and a monovalent vaccine containing 500 CCA Units of B/HK/5/72. Influenza hospitalizations totaled 448; 61% (272) Flu A, and 39% (176) Flu B infections. Two parainfluenza type 3 infections were identified at Ft. Bliss. In individuals who were hospitalized and seroconverted before 2 wks. post-vaccine, it could not be adequately differentiated between vaccine and infection antibody in the absence of a virus isolation. Thirty-five mycoplasma infections were identified.

Field studies to determine the immunogenicity of Adenovirus Vaccines were conducted utilizing 360 serum pairs collected from 6 training forts. Accumulative results indicated the type 4 vaccine to be 69% immunogenic, and the type 7 was 63.6% immunogenic.

A preliminary study to determine the effectiveness of Charcoal Viral Transport Media (CVTM), and bentonite media for transporting virus specimens was conducted using Tryptose Phosphate Broth (TPB) as a control. The recovery of Vaccinia, ECHO 9, Coxsackievirus, Polio, Adenovirus, Herpes, Mumps, and Influenza were similar, but neither media maintained virus titers as adequately as TPB.

Studies to determine whether Coxsackievirus is a significant cause of upper-respiratory infections revealed that this agent represented 25%, 36/144, of the total number of viruses isolated during the period July thru December. Serological data confirms Coxsackievirus A21 as a significant cause of ARD.

During an 8 month period of FY 77, 77 male and female military patients were screened for penicillinase producing N. Gonorrhoeae (PPNG). These individuals were selected from Far East arrivals and local area treatment failures. One PPNG was isolated; 3 other penicillin resistant gonococci were isolated, however, they were beta-lactimase negative.

A total of 115 E. Coli that were cultured from 23 acute gastroenteritis infants were serotyped and tested for enterotoxin production. The purpose of this study was to correlate the occurrence of sero-pathogenic E. coli in gastroenteritis infants, with the presence of enterotoxin. Isolates totaling 137 and 98 from other clinical and non-clinical specimens respectively showed 37 and 26 enteropathogenic E. coli respectively, but no enterotoxin was identified.

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FOREWORD

This progress report describes the efforts of this study group to forecast, detect and prevent acute respiratory diseases (ARD) and infectious diseases among military personnel. Our greatest attention has been directed towards surveillance, and prophylactic means of preventing diseases before they gain in-roads to troop training units. Therefore, emphasis has been focused on those diseases that (1) cause the greatest loss of basic combat training days among recruits, and (2) those that cost or have the potential of costing the government the largest amount for hospitalizations, e.g., Adenovirus infections, Influenza, Cocksackievirus, Meningococcal Meningitidis, urethritis and enteric pathogens. It is intended that the variety of approaches used in accomplishing these investigations will suggest pathways for further and even more efficacious studies.

We owe profound thanks to individuals in the Virology and Bacteriology Reference Laboratories at Fort Baker, California, and the Health and Environment Activity at Fort Ord, California, for their valuable efforts in specimen collections and technical assistance. Very special gratitude is owed to our Secretary, Mrs. Joy Griffin, who endured the unenviable tasks of assisting in the preparation of tables and figures, typing drafts and this final manuscript.

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ACUTE RESPIRATORY DISEASE SURVEILLANCE

INTRODUCTION

The principal cause of morbidity in military training units continues to be acute respiratory diseases (ARD), though the etiologic agents vary. Surveillance studies as conducted during the past ARD season, and for approximately 14 years before at CONUS training forts, have revealed the first signs of on-coming epidemics. These early warnings have been the indicators for vaccine administration, our most valuable prophylactic tool. On the average, 25 to 30% of the 70-85% of basic combat trainees (BCTs) who experience acute upper respiratory disease symptoms become ill enough to be hospitalized (1). These surveillance studies were performed on BCTs who were hospitalized with acute respiratory disease at 7 BCT forts, 1 AIT fort, and 1 Naval Training Center. The threat of an A/Swine like influenza epidemic encouraged a special influenza surveillance program which was conducted at 5 training forts during the year (2).

MATERIALS AND METHODS

Basic combat trainees hospitalized with ARD, totaling 6,897 at 9 training forts (including a Navy training center) throughout CONUS were subjects for this surveillance. Within 12 hours following admission, retro-uvula swabs and acute-phase sera were obtained from each individual. Convalescent sera were collected 14 to 21 days later. Because of transfers, short discharges, AWOL's, etc., paired sera were obtainable on only 2,695 individuals. Each swab was placed in charcoal viral transport media and held at 5°C (usually two-four days) until examined at the Medical Laboratory. An additional 10 throat-wash samples (utilizing trypticase soy broth) per week were collected from hospitalized patients at Forts Wood and Bliss. These were quick-frozen immediately after collecting, and shipped frozen to this laboratory. These served as special influenza surveillance samples. The same kind of Flu surveillance was conducted at Forts Knox and Dix by WRAIR. Virus isolations were accomplished in tissue culture, utilizing human embryonic (HEK) and rhesus monkey embryonic kidney (MEK) monolayers, and embryonated chicken eggs. Study sera received as pairs after the convalescent collections, were used for detecting diagnostic rises in antibody titer by the complement fixation test to adenovirus, influenza A and B, and mycoplasma. Adenovirus serotypes were identified by microtiter neutralization tests utilizing HELA cell cultures. Influenza isolates were tested for by guinea pig erythrocyte hemadsorption, and serotypes were identified using specific antisera in hemagglutination inhibition tests. Mycoplasma infections were identified by seroconversion;

isolations and species determinations were accomplished only in specifically requested cases, or to furnish information for another study. Other viral serologies and isolation techniques were employed when indicated by disease outbreaks, unusual isolates or for other diagnostic aids.

RESULTS AND DISCUSSION

Figure 1 indicates acute respiratory disease hospitalization rates per hundred per week during FY '77 at 8 training forts. The highest rates during a single week occurred at Forts Dix and Wood, 5/100/week the 3rd week in February. Forts Knox and Jackson experienced ARD rates of as high as 4/100/wk. All other forts remained below 3/100/wk. Adenovirus 4 and 7 vaccines were administered continuously throughout the year at Fort Leonard Wood. These vaccines were administered September through April at Fort Dix, and October through April at other BCT forts. Influenza vaccine was administered from the 1st week in October to the 2nd week in December at all of the training forts until the Guillion Barré Syndrome forced a discontinuance. Flu Vaccine was administered again beginning the 2nd week of February after the moratorium. Flu vaccine received by the military was bivalent A/Victoria/3/75(H₃N₂) - A/New Jersey/8-9/76(H₅N₁) 400 CCA units each; and monovalent B/Hong Kong/5/72 500 CCA units.

The ARD hospitalization rate per hundred per month, and etiologic agents at Fort Dix during FY 77 are indicated in Figure 2. The same information is shown for FY '76 on this figure for comparison. Fort Dix was one of the hardest hit BCT posts; weekly ARD hospitalizations reached 5/100/wk. A peak rate of approximately 3.2 per hundred per month was reached in February, caused mostly by influenza. The ARD peak rate reached only 2/100/mo during FY '76, and this occurred in June. Adenovirus 4 and 7 vaccines were administered September thru April, and influenza vaccines were given twice as indicated. During the year 22% ARD hospitalizations were caused by adenovirus infections (393 Adeno 21's, 25 Adeno 4's, 6 Adeno 7's, 2 Adeno 2's), 6.9% were caused by influenza (77 A's, 126 B's), 1% by Mycoplasma (18), 1 individual was hospitalized with ECHO virus, and 70% ARD was not determined.

As indicated on Figure 3 the peak ARD hospitalization rate of 3.1/100/month was reached in February at Fort Knox. The highest rate of 2/100/mo occurred last year in February also. Adenovirus 4 and 7 vaccines were administered October thru May, and influenza vaccines were given October thru December (flu vaccine was not given again after the Guillion Barré Moratorium). During the year 27.7% ARD hospitalizations were caused by Adenoviruses (321 Adeno 21's, 75 Adeno 7's), 0.4% were caused by Influenza (7 A's, 6 B's), 1.1% by Mycoplasma (1) and poliovirus (22), and 70.8% of ARD hospitalization could not be determined.

Fort Leonard Wood was one of the hardest hit BCT posts; weekly ARD hospitalizations reached 5/100/wk in February due mostly to influenza. This translated to approximately 4/100/month. Last year a peak rate of 3.5/100/mo was reached. Adeno 4 and 7 vaccines were administered continuously throughout the year. Influenza vaccine was given at two time periods as indicated in Figure 4. Adenoviruses (91 Adeno 21's, 3 Adeno 7's, 1 Adeno 4) caused 9.5% of ARD hospitalizations, Influenza (14 A's, 72 B's) caused 3.6%, polio (24), Herpes Simplex (1) and Mycoplasma (8) caused 0.9%, and 86% of ARD could not be determined.

ARD hospitalization rates at Fort Jackson during FY '77 and '76 were similar, 2/100/month both years as indicated by Figure 5. However, a larger number of etiologic agents were determined last year. Adenovirus 4 and 7 vaccines were administered October thru May, and Influenza vaccine was given from October to January. Adenovirus caused 11.4% of hospitalized ARD (43 Adeno 21's, 1 Adeno 3 and 1 Adeno 2), 1.4% were caused by Influenza (6 A's, 4 B's), 0.3% were caused by other agents e.g., polio (13 isolates), and Herpes Simplex Virus (1 HSV), 86.9% etiologic agents were not determined.

The ARD situation was mild at Fort Gordon during the year as indicated by Figure 6. The highest rate of 1.2/100/mo occurred in March. Adenovirus 4 and 7 vaccines were administered October to May, and the Influenza vaccine October to December. Of the ARD hospitalizations that occurred mostly in March, 12.3% were caused by Adenovirus 21's, 3% by Influenza (22 A's, 9 B's), 1.5 % other agents (1 Myco, 1 HSV, 9 Polio), and 83.2% could not be determined.

Fort Bliss had sporadic ARD hospitalization rates as indicated by Figure 7, but they did not peak above 1.5/100/mo which occurred in February. Adenovirus 4 and 7 vaccines were administered between October and May, and Influenza vaccines were given from October to December. Adenoviruses (52 Adeno 21's, 1 Adeno 7, 12 Adeno 4's, 3 Adeno 3's) caused 12.8% ARD hospitalizations, 10.9% were caused by Influenza (49 A's, 39 B's), 0.5% by other agents e.g., 2 HSV, 1 vaccinia, 10 polio and 1 mycoplasma, and 75.7% of causes could not be determined.

As Figure 8 indicates ARD hospitalization rates were low during the year at Fort Sill. However, there were 15 Influenza A infections. Adenoviruses (14 Adeno 21's, 1 Adeno 7) caused 10.8% of ARD hospitalizations, 89.2 etiologic agents could not be determined. ARD rates did not move above 0.6/100/month. Vaccines, Adenovirus 4, 7 and Influenza were given as indicated by Figure 8.

Fort Ord was the only AIT Fort that was surveyed. As indicated in Figure 9 the ARD hospitalization rate only reached 0.7/100/month in February. Adenoviruses (3 Adeno 21's) caused 2.2% of these hospitalizations, 1% were caused by Influenza (1 A, 1 B), 4.5% by other agents (1 Mycoplasma, 2 HSV, 1 polio), and 76.5% ARD was not determined. Influenza vaccine was administered during November and December.

Male and Female BCT was conducted at Fort Jackson during the year. As indicated in Table 1, 19% of isolation samples and 26% of serum pairs received were from Female BCT's. Agents that were isolated by month are shown. Isolations from Female BCT's totaled 12 Adenovirus 21's and 4 polio viruses. Influenza was not evident in female trainees during the year.

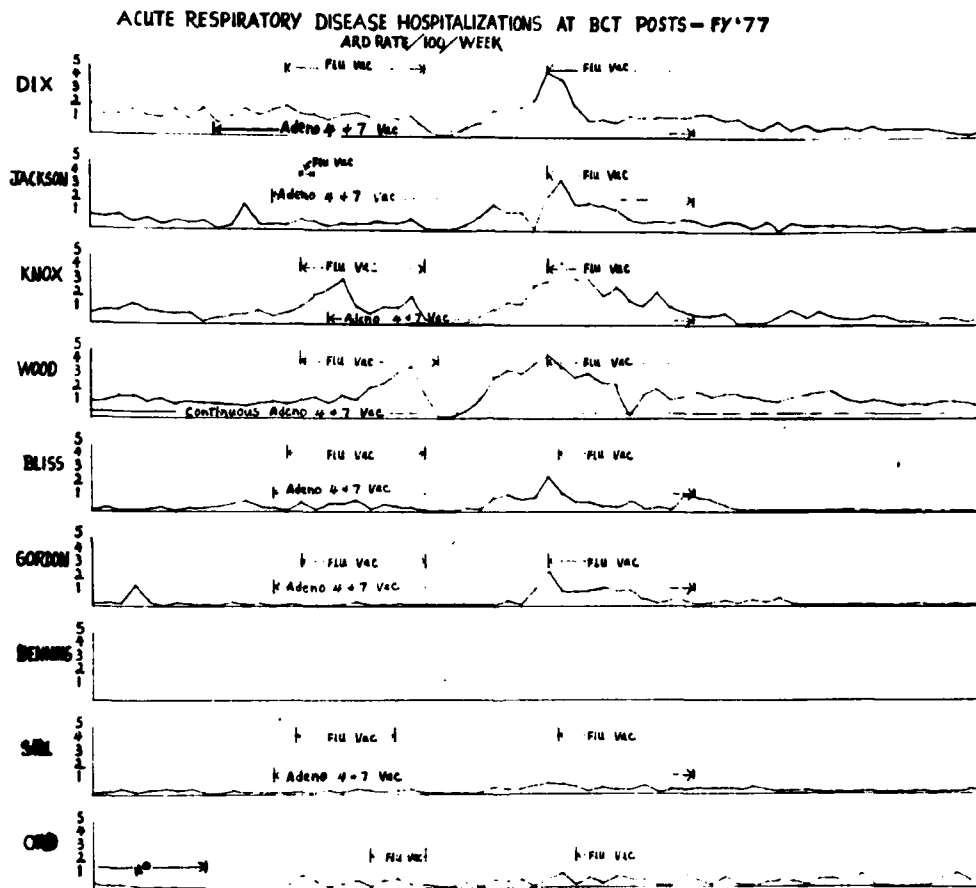
SUMMARY

Figure 10 is a summary of the total ARD picture among all BCT's, and indicates the average ARD hospitalization rate per hundred per month for the 8 forts that were studied during FY '77. FY '76 is shown at the top of this figure for comparison. The highest average ARD hospitalization rate per hundred per month was approximately 2.7 in February. A low number of influenza admissions occurred throughout the year but mostly between December and May. Most stations administered adenovirus vaccines 1 October 76 through 1 May 77. Including all of the forts, an average of 19.9% of ARD was caused by the adenoviruses, 4.5% by influenza, 0.9% by other agents e.g., mycoplasma, HSV and polioviruses; and 74.7% were caused by agents that were not determined. Table 2 summarizes causative agents by isolations and seroconversions obtained out of the total samples taken from individuals hospitalized with ARD at the eight training forts and one Naval Training Center that were surveyed. From 6,897 individuals, 1,237 virus isolations were made (17%). Of the isolations, 1,073 (86%) were Adenoviruses, and 941 (87.7%) of the Adenoviruses were type 21. The number of Influenza isolates were small (8 Flu A's, 11 Flu B's). Various other isolates can also be seen in Table 2. Of the 2,695 serum pairs studied, 966 (35%) indicated seroconversions. Four hundred and eighty three (50%) seroconversions were to Adenoviruses, 176 (18%) to Influenza A, 272 (28%) to Influenza B, and 35 (3%) were to Mycoplasma. The Influenza agents were A Victoria and B Hong Kong. It could not be determined if 130 polio isolates were vaccine virus or those that actually caused infections.

REFERENCES

1. Smith, Creed D., et al: Prophylactic Methods in the Prevention of Diseases Among Army Personnel. Annual R & D Report-DA Project 3A061102B71Q, 1971 thru 1976.
2. Smith, Creed D., Henry A. Leighton and Ronald S. Shiromoto: Antigenicity and Reactivity of Influenza A/New Jersey/8/76 Virus Vaccines in Military Volunteers at Fort Ord, California. J. Infect. Dis. 131: Supplement, S460-S465, 1977.

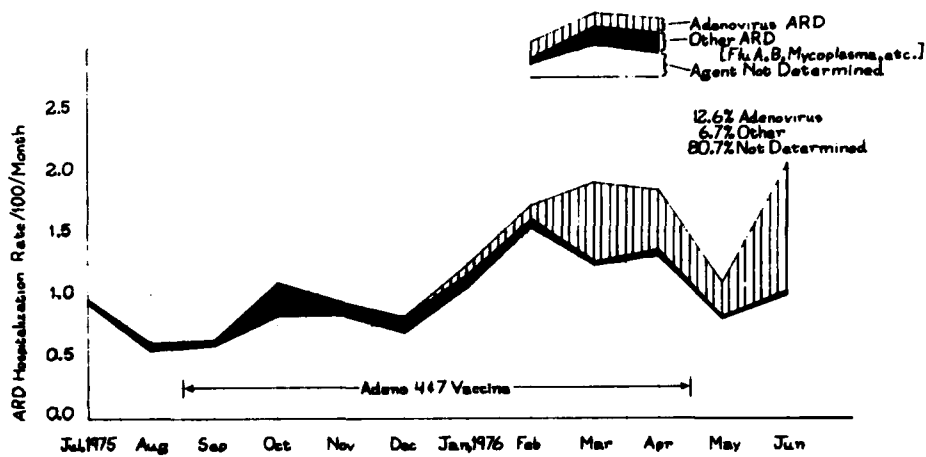
FIGURE 1.



Aug 76 Aug 76 Sep 76 Oct 76 Nov 76 Dec 76 Jan 77 Feb 77 Mar 77 Apr 77 May 77 Jun 77 July 77 Aug 77 Sept 77

FIGURE 2.

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Dix - Fiscal Year 1976



Acute Respiratory Disease Hospitalizations with Adenovirus and Influenza Infections
Fort Dix - Fiscal Year 1977
(includes 1 Quarter)

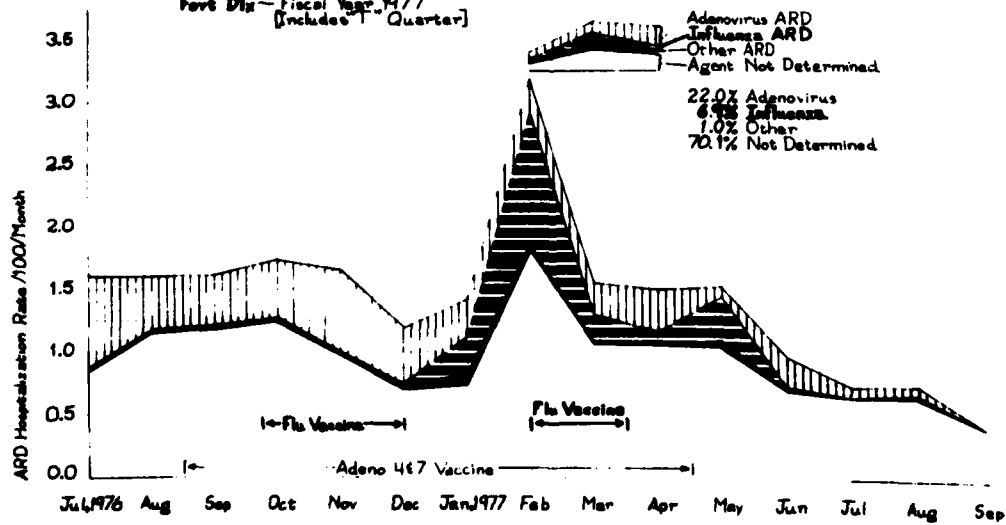


FIGURE 3.

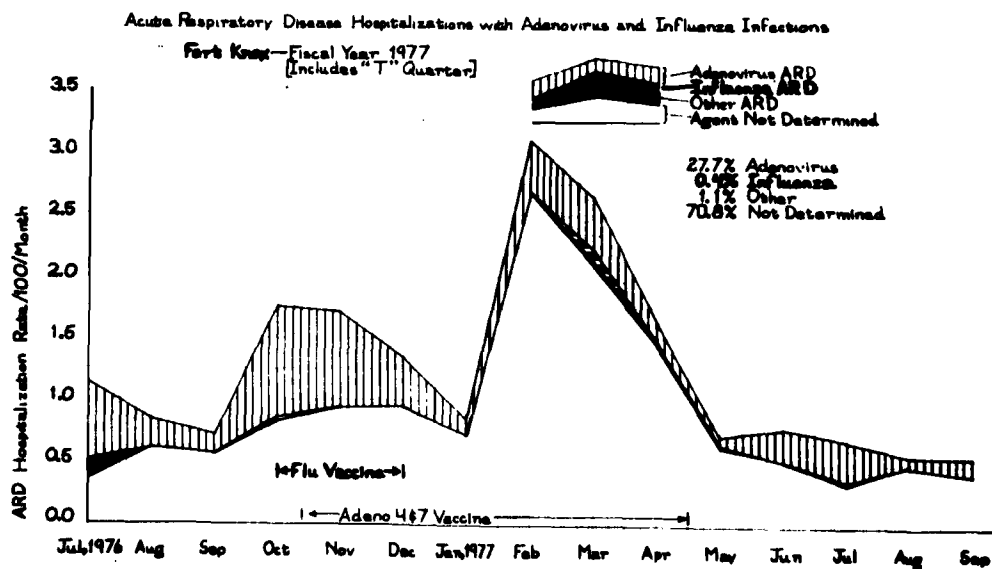
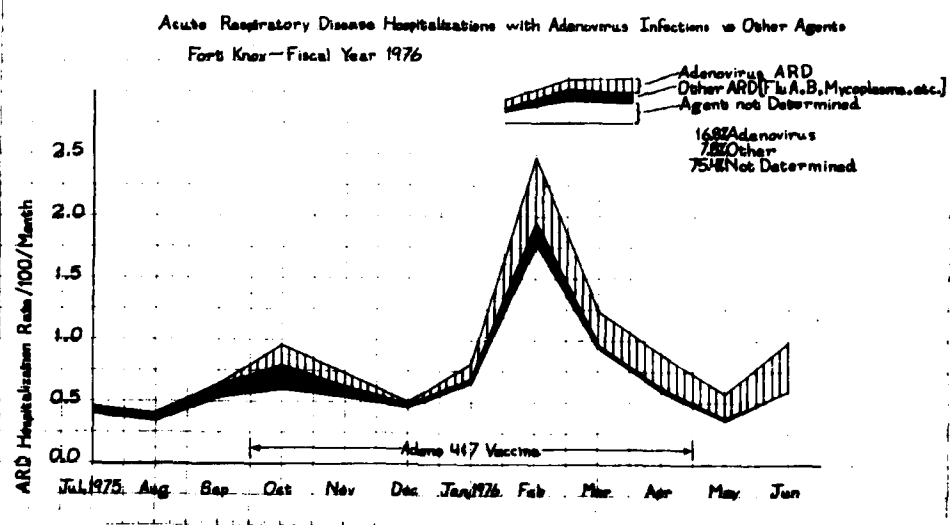


FIGURE 4.

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents

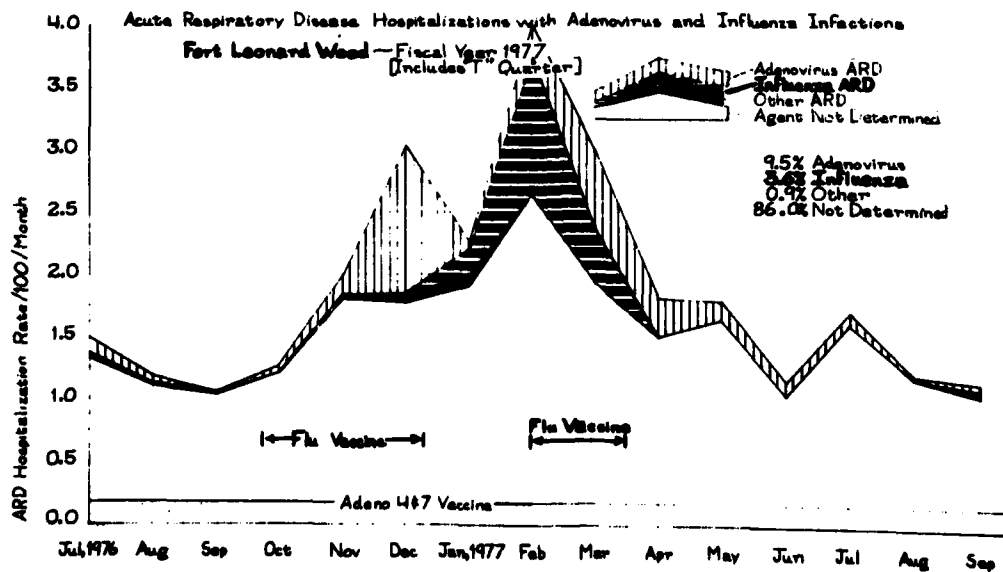
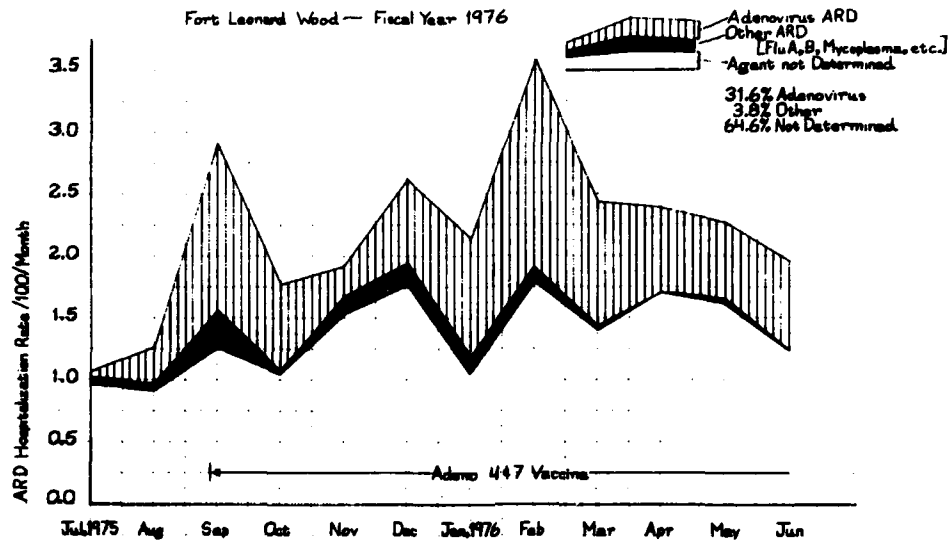


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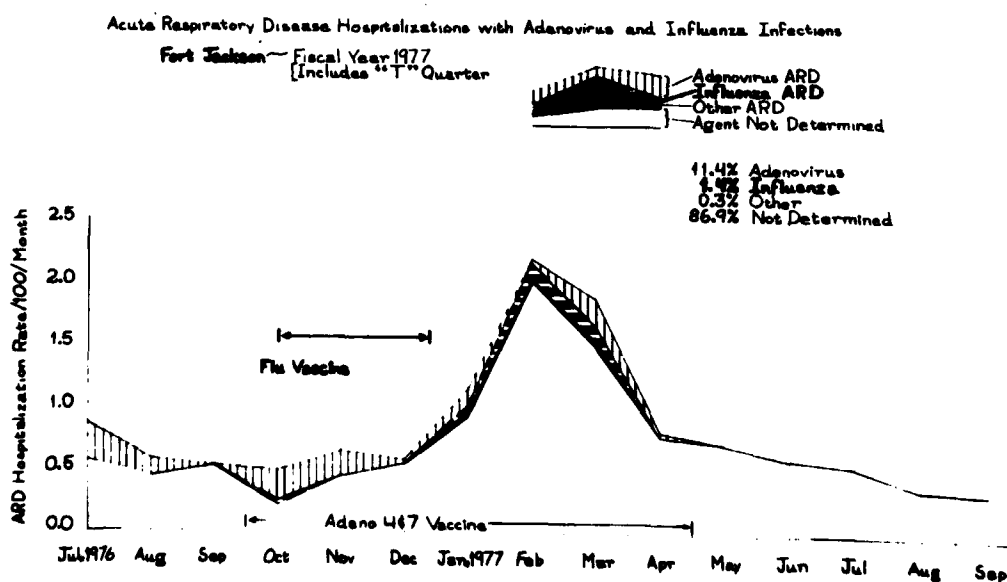
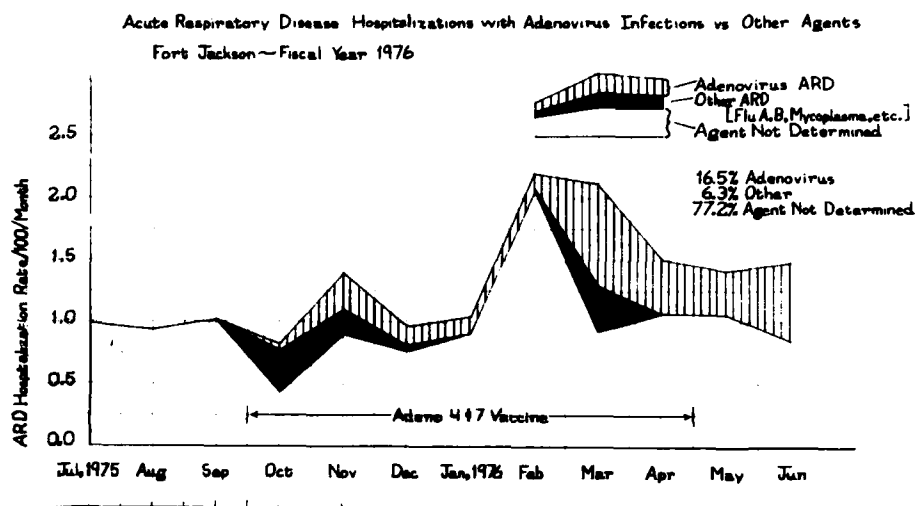


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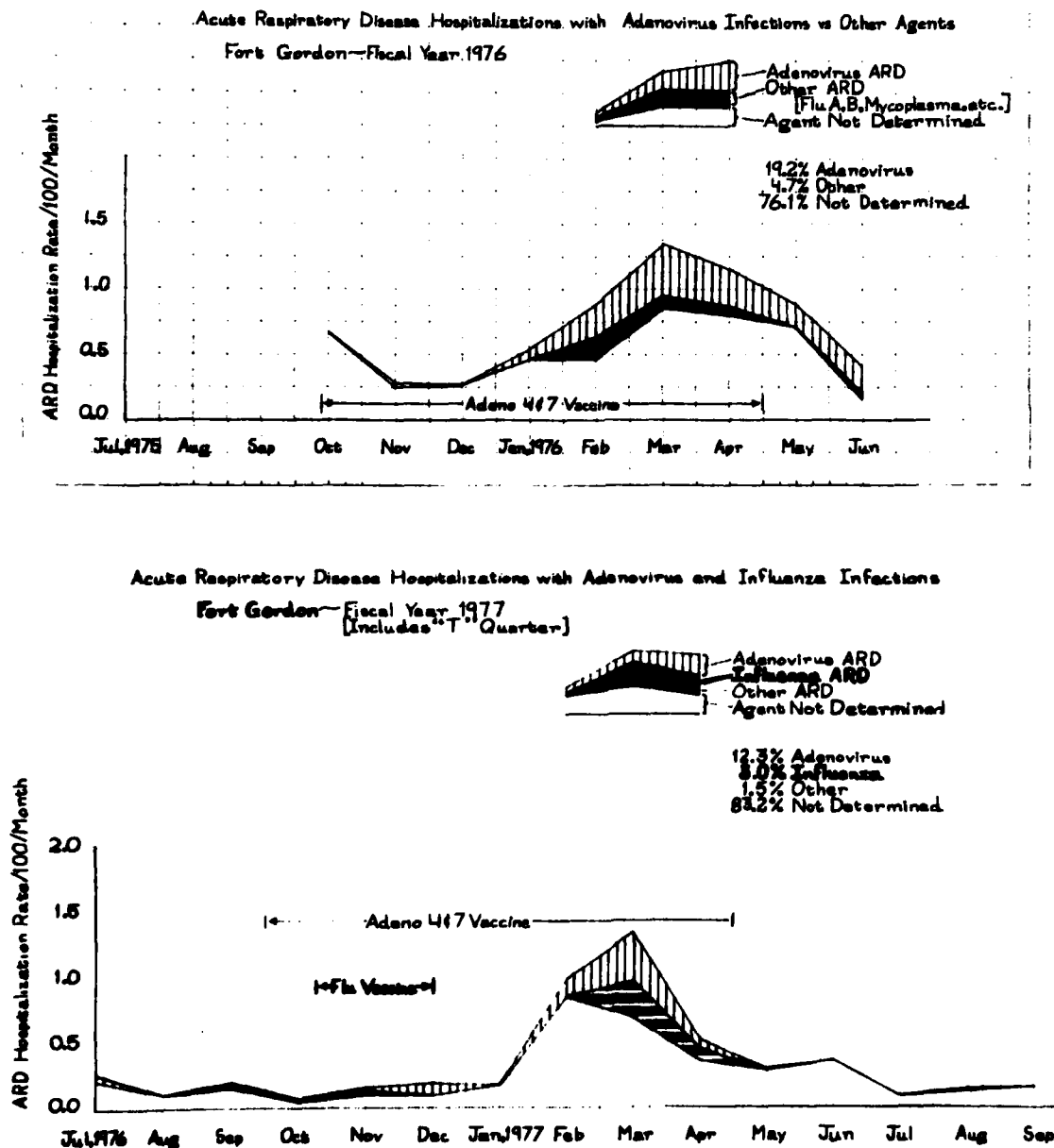


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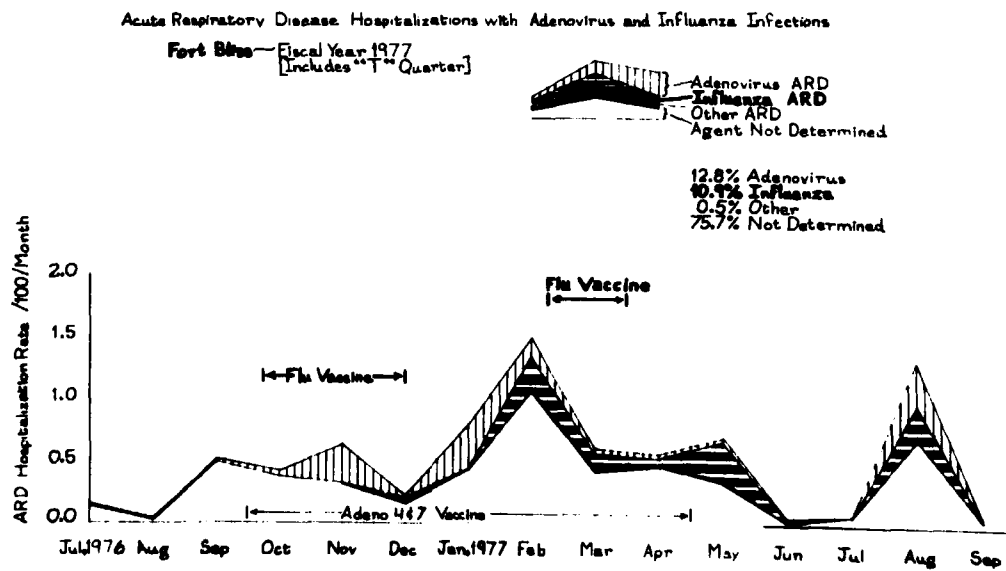
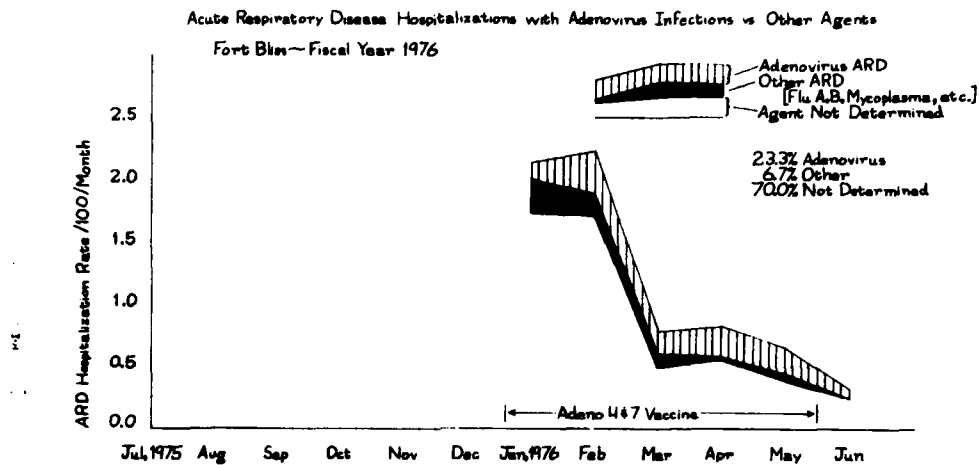


FIGURE 8.

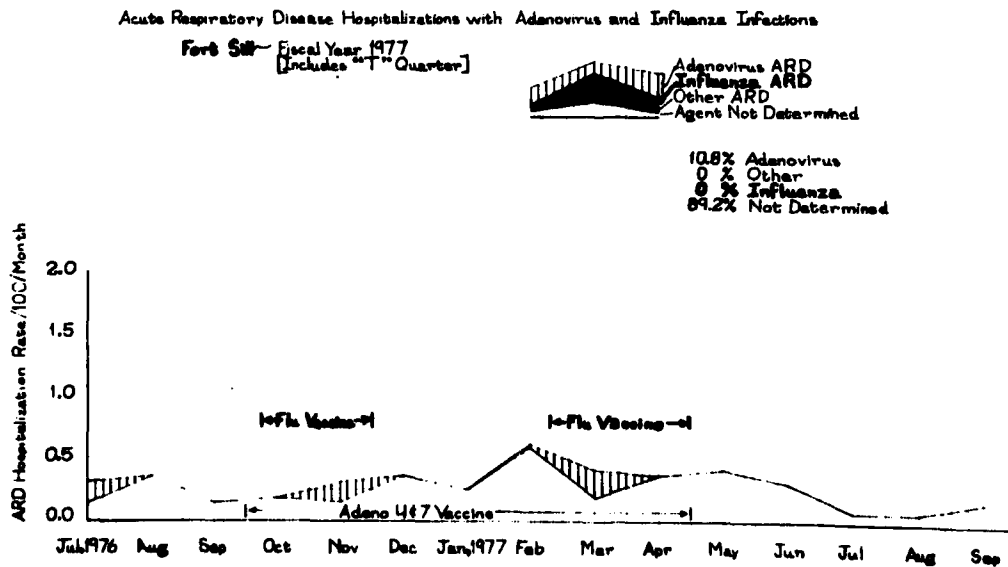
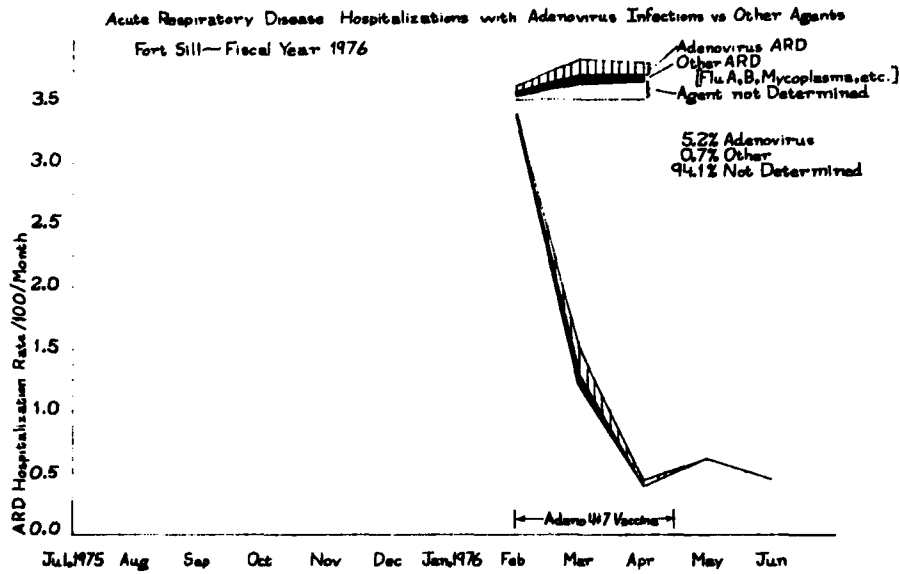
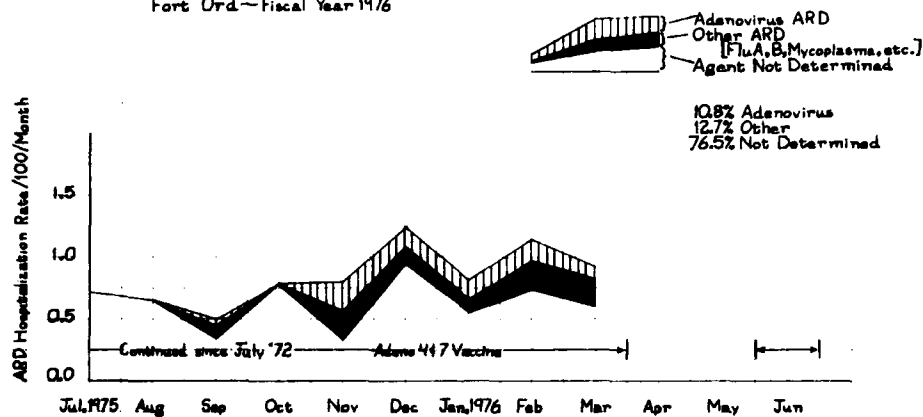


FIGURE 9.

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Ord - Fiscal Year 1976



Acute Respiratory Disease Hospitalizations with Adenovirus and Influenza Infections
Fort Ord - Fiscal Year 1977
[Includes 4th Quarter]

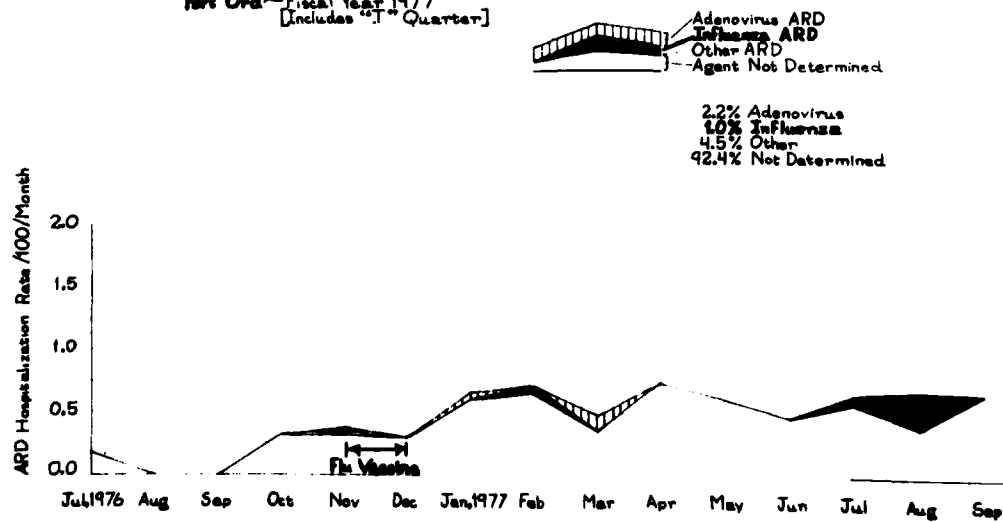


Table 1.

ETIOLOGY OF ARD IN HOSPITALIZED FEMALE
MILITARY PERSONS AT FORT JACKSON

JULY 1976 - SEPTEMBER 1977

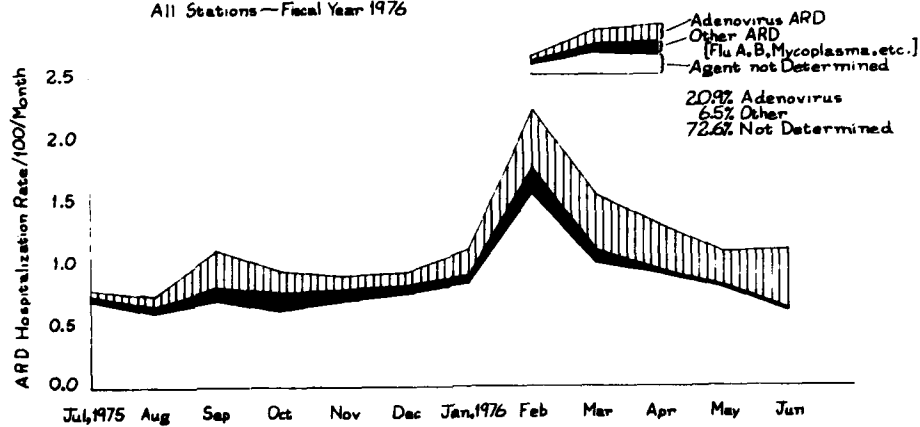
ISOLATIONS										SEROCONVERSIONS					
MONTH	TOTAL SPEC RECD ♀ ♂	Ad 4	Ad 7	Ad 21	Polio	Flu A	Flu B	Myco	TOTAL ISOL.	% ISOL.	TOTAL PRS RECD ♀ ♂	ADENO <1wk >2wk	FLU A <1wk >2wk	FLU B <1wk >2wk	MYCO
JULY	5 6			1	1				2	40%	1	2			
AUG	4														
SEPT															
OCT	12 58			4					4	33%	9	41	5		
NOV	5 25			4					4	80%	3	15	1		
DEC	3 21				1				1	33%	3	3			
JAN	12 39			2	2				4	33%	5	11	2		
FEB	4 53									0	1	6			
MAR	17 72			1					1	5%	3	8			
APR	9 30									0	2	2			
MAY	2 20									0					
JUN															
JUL															
AUG	1 18							1	1	100%	1	7			
SEPT	5 44									0					
TOTALS	75 390			12	4			1	17	23%	25	95	8 (32%)		
(♀) 19%															
(♀) 26%															

(♀) 19%

(♀) 26%

FIGURE 10.

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
All Stations—Fiscal Year 1976



Acute Respiratory Disease Hospitalizations with Adenovirus and Influenza Infections
All Stations—Fiscal Year 1977
[Includes "T" Quarter]

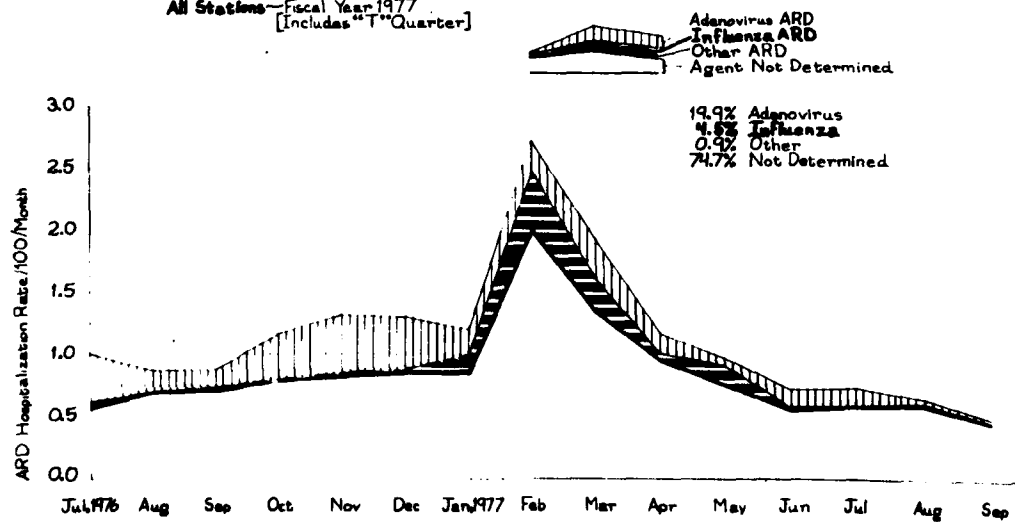


TABLE 2.

1 JULY 76 - 30 SEPT 77

STATION	TOTAL ISOL SPEC	ISOLATIONS										PARA FIRST APPEARED	TOTAL PAIRS RECD.	SEROCONVERSIONS				MYCO PNEU			
		Ad 2	Ad 3	Ad 4	Ad 7	Ad 21	VAC	POLIO	FLU A	FLU B	HSV			ECHO	ADENOVIRUS <1WK >2WK	FLU A <1WK >2WK	FLU B <1WK >2WK				
DIX	2296	2		25	6	395		44	2	10		1	7 JAN 76	952	47	190	21	56	68	58	18
KNOX	1545				75	321		22					8 OCT	148	5	62	2	5	2	4	3
ORD AIT	129					3		1			2		10 MAR	84	3			1		1	1
JACKSON	354	1	1			43		13		1	1		11 FEB	90	4	16	3	3	3	1	
GORDON	298					22		9	3		1		28 JAN	195	2	26	9	13	3	6	1
WOOD	1282			1	3	91		24			1		12 NOV	888	48	49	7	7	26	46	8
SILL	95				1	14		3					10 MAR	50		4	2	13			
BLISS	551		3	12	1	52	1	10	3		2		21 JAN	279	5	52	17	32	18	21	1
USN	348			1				4			4			9							3
TOTALS	6897	3	4	39	86	941	1	130	8	11	11	1	2	2695	111	372	59	117	122	150	35

28.373.68.087.7

44%

56%

% .28 .37 3.6 8.0 87.7

44%

56%

<1 = 0 and 1ST WEEK

>2 = 2ND WEEK and ABOVE

ANTIGENIC EVALUATION OF ADENOVIRUS VACCINES FY '77

DESIGN:

Antigenic evaluations of Type 4 and 7 Adenovirus Vaccines were conducted during FY '77 on the first recruits who received the new vaccine lots distributed to Forts Knox, Sill, Dix, Bliss, Wood and the EPMU-5, US Navy San Diego. No sera relevant to this study were received from Forts Jackson, Gordon and Ord. Serum neutralizing antibody titers were determined at this laboratory on 180 pairs consisting of pre-vaccine and 3 week post-vaccine samples. The micro-titer homologous neutralization test which is used routinely in this laboratory was employed.

RESULTS:

As indicated in Table 1, Seroconversion rates ranged from 100% to 25% for Adenovirus type 4 Wyeth Lot #OPL-24 Vaccine, and from 78.6% to 41.2% for Adenovirus type 7 Wyeth Lot# 55142 Vaccine. The accumulative results at the bottom of Table 1 indicate that the Adenovirus type 4 Vaccine was 62.8% immunogenic, and the type 7 was 67.2% immunogenic.

TABLE 1
ANTIGENIC EVALUATION OF ADENOVIRUS VACCINES FY '77

<u>FORT KNOX</u>			
	<u>Pairs Tested</u>	<u>Conversions/Susceptibles</u>	<u>Percent Conversions</u>
Adenovirus 4, Wyeth Lot #OPL-24	72	29/48	60.4
Adenovirus 7, Wyeth Lot #55142	72	34/50	68.0
<u>FORT SILL</u>			
Adenovirus 4, Wyeth Lot #OPL-24	20	8/16	50.0
Adenovirus 7, Wyeth Lot #55142	20	10/13	76.9
<u>FORT DIX</u>			
Adenovirus 4, Wyeth Lot #OPL-24	25	18/18	100.0
Adenovirus 7, Wyeth Lot #55142	25	11/14	78.6
<u>FORT BLISS</u>			
Adenovirus 4, Wyeth Lot #OPL-24	22	11/14	78.6
Adenovirus 7, Wyeth Lot #55142	22	7/14	50.0
<u>FORT WOOD</u>			
Adenovirus 4, Wyeth Lot #OPL-24	18	1/4	25.0
Adenovirus 7, Wyeth Lot #55142	18	7/17	41.2
<u>EPMU-5, US NAVY, SAN DIEGO</u>			
Adenovirus 4, Wyeth Lot #OPL-24	23	11/13	84.6
Adenovirus 7, Wyeth Lot #55142	23	8/13	61.5
<u>FORTS JACKSON, GORDON, AND ORD</u>			
- No Sera Received -			
<u>ACCUMULATIVE RESULTS</u>			
Adenovirus 4 Vaccine	180	78/113	69.0
Adenovirus 7 Vaccine	180	77/121	63.6

% Susceptible to Adenovirus 4 = 62.8
% Susceptible to Adenovirus 7 - 67.2

INVESTIGATIONS TO DETERMINE IF COXSACKIE A21 VIRUS
IS A SIGNIFICANT CAUSE OF ACUTE RESPIRATORY
DISEASE AMONG ARMY RECRUITS

INTRODUCTION

The Adenovirus Surveillance Program was established to monitor the presence of infectious agents of acute respiratory diseases (ARD) in basic combat trainees (BCT's). In 1973 adenovirus infections represented 47.9% of all ARD, and agents of unknown etiology, 36%. In 1974, primarily due to the production and use of highly immunogenic Adenovirus 4 and 7 vaccines, the percentage of adenovirus infections was reduced to 9.5%. However, the rate of infections due to agents of unknown etiology rose sharply to 68.0%(1).

This study group has observed that Cocksackie A21 Virus has caused sporadic epidemics of ARD in basic training centers, and in the winter of 1966 played a significant role as a causative agent of ARD. In the years between 1966 and 1975, however, Cocksackie A21 Virus was isolated only infrequently from basic trainees. But in fiscal year 1975, 41 cases of Cocksackie A21 Virus were isolated and identified.

It has been demonstrated that the charcoal viral transport medium (CVTM) is an excellent carrier medium for adenovirus(2), but its ability to sustain other viruses has been questioned(3). Thus the number of Cocksackie A21 Virus isolates recovered while using CVTM may not be truly indicative of the presence of A21 within a given population. Therefore, a serological procedure is needed to adequately determine if Cocksackie A21 Virus is a major contributor of ARD in basic training centers.

In order to provide background information for future studies, we performed a preliminary study to determine the prevalence of Cocksackie A21 Virus antibodies during a period when a significant number of these agents were recovered. The time selected was between July and December 1975, when 36 Cocksackie A21 Virus isolates were confirmed from various training centers.

METHODS AND MATERIALS

Between July and December 1975, five basic training forts submitted 736 throat swab specimens for isolation and 220 paired sera for serologic testing. Prototype Cocksackie A21 Virus was grown and titered in vero monkey kidney cells (VMK). All sera were titrated against 100% TCD₅₀ of Cocksackie A21 Virus. The microtiter serum neutralization test as described by Melnick(4) was used to determine the presence of Cocksackie A21 Virus antibodies. Plates were read daily and final readings were made after three days. Complement fixation tests were performed for adenovirus, mycoplasma, and Influenza A and B(5).

RESULTS AND DISCUSSION

One hundred forty-four (144) viruses were recovered from the 736 throat swabs submitted. Thirty-six viruses were identified as Cocksackie A21 Virus representing 25% of the total number of viruses isolated. Cocksackie A21 Virus was the agent most frequently isolated at Fort Knox (15/32) and Fort Dix (8/11).

TABLE 1. VIRUSES ISOLATED FROM JULY TO DEC. 1975

STATION	# OF THROAT SWABS SUBMITTED	ADENOVIRUS	%	COXSACKIE A21 VIRUS	%	POLIO	%	TOTAL # OF ISOLATES	%
WOOD	222	56	25.2	7	3.2	3	1.4	66	29.8
KNOX	202	15	7.4	15	7.4	2	1.0	32	15.8
JACKSON	91	8	8.8	3	3.3	1	1.1	12	13.2
DIX	151	0	0	8	5.3	3	2.0	11	7.3
POLK	70	17	24.3	3	4.3	3	4.3	23	32.9
TOTAL	736	96	13.4	36	4.9	12	1.6	144	19.6

TABLE 2. COXSACKIE A21 VIRUS SERUM NEUTRALIZATION VS ISOLATIONS

SERUM NEUTRALIZATION FOR COXSACKIE A21 VIRUS

STATION	# OF PAIRS SERA SUBMITTED	ISOLATES *				TITERS ≥20/≥20	≥4-FOLD RISES	≥ %- 4-FOLD RISES	GMT A/C	RATED GMT C/A
		ADENO	COX A21	POLIO	TOTAL					
WOOD	87	33	3	0	36	11	0	0	1.77/1.77	1.0
KNOX	39	3	2	0	5	1	7	18.0	1.10/2.37	2.53
JACKSON	32	2	0	0	2	4	2	6.3	1.62/2.00	1.23
DIX	33	0	1	1	2	3	3	9.1	2.01/3.0	1.49
POLK	38	8	2	2	12	8	5	13.2	2.53/3.89	1.53
TOTAL	229	46	8	3	57	27	17	7.4	1.68/2.32	1.38

* Isolates listed relate only to those patients for which paired sera were received.

Microtiter serum neutralization tests for Coxsackie A21 Virus were performed on the 229 paired sera and resulted in 177 (77.3%) pairs with titers of less than 10 for both acute and convalescent sera. If an equal to or greater than 20 Ab titer is considered to be suggestive of recent infection, there is a significant difference when comparing the antibody response to Coxsackie A21 Virus between the acute and convalescent sera (chi-square, $P < .05$). Seventeen pairs showed four-fold or greater rises in neutralizing antibody titer to Coxsackie A21 Virus; three of these pairs were from patients from whom Coxsackie A21 Virus had been isolated from throat swabs, four were from patients with either positive serology or isolation results for other agents, and 10 were from patients previously negative by serology and isolation procedures. (All paired sera received in this study were previously tested by CF for *Mycoplasma pneumoniae*, adenovirus, and Influenza A and B.) Twenty-seven additional pairs showed neutralizing antibody titers of equal-to or greater-than 20 for both the acute and convalescent sera. Of these, 17 were from patients previously positive for another agent by either serology or isolation procedures, and 10 were from patients who had previously been negative by both serology and isolation procedures.

SUMMARY

Coxsackie A21 Virus represented 25% (36/144) of the total number of viruses isolated during the period July through December, 1975. This is a significant figure when compared to previous years when few or no Coxsackie Viruses were isolated by this laboratory. Of the patients tested by serum neutralization (229), 44 had ≥ 4 -fold rises in antibody titer to Coxsackie A21 Virus, or titers which were equal-to or greater-than 20 in both acute and convalescent sera. However, of these 44 patients, 20 were not associated with a disease-causing agent by either isolation or complement fixation testing against adenovirus, *Mycoplasma pneumoniae*, or Influenzavirus A and B. Either directly or by implication, 20 patients were associated with Coxsackie A21 Virus. The 128 (55.9%) patients who were not previously associated with a disease-causing agent can be reduced to 108 (47.2%), thereby reducing the total number by 8.7%. Calculation of the ratio of geometric mean titers between the convalescent sera and the acute sera resulted in Fort Knox (2.15) and Fort Polk (1.53) having the highest ratios. These figures correlate well with Table 2, which demonstrates that the largest number of ≥ 4 -fold rises to Coxsackie A21 Virus were also at Fort Knox and Fort Polk.

For the period July through December, 1975, viral isolation and serologic studies indicate that Coxsackievirus A21 was a significant cause of upper respiratory disease among basic trainees. Further studies are now in progress to determine the prevalence of Coxsackie A21 Virus antibodies during July to December, 1977, a period when few of these viruses were isolated.

LITERATURE CITED

1. Smith, Creed D., LTC, MSC; George R. French, LTC, MSC; Henry A. Leighton, COL, MC; S. Vern Juchau, MAJ, MSC: Prophylactic Methods of Prevention of Disease among Army Personnel, Annual Report DA Project. 3A061102B71Q, August 1975 and 1976.
2. Leibovitz, Albert, COL, MSC: A Transport Medium for Diagnostic Virology, Proc. Soc. Exp. Biol. Med. 131:127-130.
3. Stability of Different Viruses in a Newly Developed Transport Media. F. R. Bishai; N.A. Labzoffsky: Canadian J. Microbiology, Vol. 20, 1974, p. 75-80.
4. Melnick, J., Wenner, J. Enteroviruses, In Edwin H. Lennette, M.D., Ph.D.; Natalie J. Schmidt, Ph. D.: Diagnostic Procedures for Viral and Rickettsial Infections, Fourth Edition, 1969, p. 589-592.
5. Edwin H. Lennette M.D., Ph. D.; Natalie J. Schmidt, Ph.D.: Diagnostic Procedures for Viral and Rickettsial Infections, Fourth Edition 1969, p. 51-57.

INVESTIGATIONS TO IMPROVE THE TRANSPORT MEDIUM USED FOR THE ADENOVIRUS SURVEILLANCE PROGRAM

INTRODUCTION

In 1969 the Leibovitz Transport Medium "CVTM"⁽²⁾ was developed. It became the basic transport medium for shipping viral specimens for the Adenovirus Surveillance Study. In fiscal year 1976, the recovery rate of adenovirus at this laboratory was 16.9%, which was compared to 23.19% sero-conversions. However, the agents of unknown etiology have increased from 36.0% to 68.9% from 1963 to the present. The total viral specimens received during this period was 2,450. Viral agents isolated were identified as follows: 415 adenoviruses, 77 polioviruses, 42 coxsackievirus A-21, 57 influenza A viruses, 2 influenza B viruses, and 2 Herpes simplex viruses. The percentage of influenza A and B viruses isolated was 2.4%. However, viral serology performed by the complement fixation technique demonstrated a 16.31% of sero-conversion rate to influenza virus.⁽¹⁾

Investigations have indicated that the CVTM is not capable of stabilizing many of the viruses which are found in the upper respiratory tract.⁽³⁾ Therefore, the need to utilize a more efficient transport medium has become increasingly evident.

In this study an ambient temperature bentonite transport media developed by F. R. Bishai and N.A. Labzoffsky⁽³⁾ was compared to the standard CVTM transport medium currently used in the Adenovirus Surveillance Study.

The mode of action of the new medium is that bentonite will act as a cation exchange resin as well as a good absorbing agent for electropositive and electronegative proteins⁽⁴⁾. The result will be the preservation and stabilization of viral particles.

METHODS AND MATERIALS

The original protocol called for approximately 500 specimens to be taken from one to two stations which appeared to have a large number of non adenovirus acute respiratory disease. Specimens were placed in Bentonite Transport Medium (BTM), Charcoal Viral Transport Medium (CVTM) and Tryptose Phosphate Broth with gelatin (TPB). Before this extensive and costly project was initiated, a preliminary study was performed to determine the merits of such a study. Eight representative most commonly isolated viruses were selected to determine the effectiveness of the three viral transport media. Each virus was pre-titered in cell cultures to ensure infectivity. Eighteen sterile cotton swabs were placed into a screw-capped jar containing a specific virus. After absorption of the fluid, one swab was placed into each of ten tubes containing BTM, CVTM, and TPB. The tubes containing bentonite and charcoal viral transport media were held at ambient temperatures, and the tryptose phosphate broth

media was frozen at -60°C . One swab from each media was titered in cell cultures at 1, 5, 7, 9, 14 and 21 day intervals. The presence of the myxoviruses was determined by hemadsorption test with guinea pig red blood cells after five days of incubation at 37°C . The presence of the remaining viruses was determined by microscopic observation of viral cytopathogenic effect. All cultures were examined daily and discarded at the end of seven days.

RESULTS

The recovery of vaccinia, ECHO 9, Coxsackievirus A-21, Polio, and Adenovirus 21 were almost identical for the three viral transport media studied (Table 1). The bentonite and CVTM media were similar for the recovery of herpes, mumps and Influenza A/HK, but compared to the Tryptose Phosphate Broth media, neither media maintained viral titers adequately.

SUMMARY

The preliminary study to determine the effectiveness of CVTM, bentonite, and tryptose phosphate broth viral transport media indicates that bentonite is no more effective than CVTM in the maintenance of viral agents. It is also evident that tryptose phosphate broth is superior to bentonite and CVTM in sustaining labile viruses such as herpes and the myxoviruses. The data resulting from this preliminary study precludes further investigations utilizing these same media.

REFERENCES

1. Smith, Creed D., LTC, MSC; Robert S. Stewart, 1LT, MSC, Ronald S. Shiromoto, DAC 12, Angus C. Hull, DAC11. Prophylactic methods in prevention of disease among army personnel, Annual Report, August 1976.
2. Leibovitz, A., A transport medium for diagnostic virology. Proc. Soc. Esp. Bio. E. Med., 131:127-130, 1969.
3. Bishai, F. R. and Labzofsky, N. A. Stability of different viruses in a newly developed transport medium. Can. J. Micro. Vol. 20, 1974, pp. 75.
4. Oker-Blom, N. and Nikkila, E. 1955, Bentonite purification of certain viruses. Ann. Med. Exp. bio. Fenn. 33:190-199.

TABLE 1

SURVIVAL OF VIRAL AGENTS IN VARIOUS TRANSPORT MEDIA OVER
A 21-DAY STORAGE PERIOD

	TRANSPORT MEDIA (days in storage)																	
	<u>BENTONITE</u>						<u>CVTM</u>						<u>TRYPTOSE PHOSPHATE BROTH</u>					
	1	5	7	9	14	21	1	5	7	9	14	21	1	5	7	9	14	21
Vaccinia	2 ^a	2	2	1	2	2	2	2	2	2	1	2	2	2	2	1	2	2
ECHO 9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Herpes	2	2	0	0	-- ^b	--	2	0	0	--	--	--	2	2	2	2	--	--
Influenza A/HK	1	1	-	-	--	--	0	1	-	--	--	--	2	2	0	0	--	--
Coxsackie A21	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Mumps	3	1	0	0	--	--	3	3	0	0	--	--	3	2	3	3	1	1
Poliovirus	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Adenovirus 21	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

a. Inverse logarithm to the base 10 of the highest dilution to yield discernible viral growth within 5 days.

b. No discernible viral activity within 5 days.

RAPID DIAGNOSIS OF UPPER RESPIRATORY VIRUSES IN CELL CULTURE UTILIZING THE DIRECT IMMUNOFLOUORESCENCE PROCEDURE

INTRODUCTION

Present cell culture procedures for virus isolation and identification in the diagnostic laboratory require six days or longer to definitively identify an etiologic agent. Fluorescence antibody procedures have been demonstrated to be a rapid method for the identification of various viral agents (1, 2, 5). Several approaches utilizing the immunofluorescence procedure have been attempted here at Fort Baker, but with limited success. Specifically, these were the use of the indirect FA procedure and the use of ciliated epithelial cells from nasopharyngeal smears(3).

Several papers have reported success in the diagnosis of influenza by staining of ciliated epithelial cells (1, 6, 7). However, the specificity of their data has been questioned(8), and more experience with staining of nasopharyngeal smears must be accumulated. The initial research application submitted pertained specifically to the indirect immunofluorescence examination of nasopharyngeal smears. However, the question of specificity in working with nasopharyngeal smears and the recent availability of commercially produced specific conjugates to a variety of upper respiratory agents have necessitated a change in protocol. The emphasis will be placed on the use of direct immunofluorescence procedures, which will result in clearer staining and better resolution of morphological localization of viral antigens(4). The utilization of prepared infected cell cultures will also provide the technologists with a substrate in which the level of infectivity can be regulated and a test into which proper quality control measures can be incorporated.

METHODS

Specific Fluorescein conjugates were purchased from Microbiological Associates and Flow Laboratories for adenovirus, Influenzavirus A and B, Parainfluenza 1, 2, 3, Respiratory syncytial, mumps, cytomegalovirus and Herpes I and II. Fluorescein-conjugated viral immunoglobulins to be used for detecting viral antigens in clinical material or for identification of viral isolates must be titrated in order to select an optimal "working dilution." Serial dilutions of the conjugates were tested on fixed cell cultures of prototype virus-infected cells and uninfected cells of the same lot. The working dilution is the highest dilution producing 3+ to 4+ staining intensity with the virus-infected cells with no staining of uninfected cells. Throat swab specimens will be collected and placed in charcoal viral transport medium (CVTM) or tryptose phosphate broth with gelatin (TPB). The CVTM may be shipped at ambient temperatures, but the TPB must be immediately frozen and shipped on dry ice to this Reference Laboratory. This will act as a control. These specimens will be inoculated into all cultures upon receipt and will be observed for

viral activity.

The direct immunofluorescence procedure will be performed on all positive cultures suspected of adenovirus, influenza A and B, parainfluenza 1-3, or herpes, mumps, CMV, or rubeola virus infection. The direct FA procedure will be augmented by heterologous serum neutralization in cell cultures.

RESULTS AND DISCUSSION

(Chart)

<u>Conjugate</u>	<u>Manufacturer</u>	<u>Lot #</u>	<u>AG Source</u>	<u>Cell</u>	<u>Titer</u>
Herpes I	Flow	R848103F	ATCC	Flamnion	1:10
Herpes II	"	R848205F	"	"	"
Paraflu I	"	R821202F	Mt Zion	PMK	"
Paraflu II	"	R822102F	"	"	"
Paraflu III	"	R822102F	"	"	"
Influenza A	"	R825101F	CDC	"	1:15
Influenza B	"	R826101F	"	"	"
CMV	"	R846101F	Mt Zion	WI38	1:10
Mumps	"	R828101F	CDC	PMK	1:20
Adenovirus 5	"	R860501F	ATCC	Flamnion	1:10
Respiratory Syncytial	"	R830001F	ATCC	"	1:20
Adenovirus	MBA	3-7767	ATCC	"	1:5
Herpes Bivalent	"	3-7716	ATCC	"	1:5
CMV	"	3-7721	Mt Zion	WI38	1:5

The fluorescein conjugates from Flow Laboratories were of excellent quality, particularly the mumps, parainfluenza 1, 2, 3, and Influenza A. The Herpesvirus 1 and 2 conjugates both titered 1:10; however, differentiation between the two viruses was very subjective. The CMV fluorescence was distinctive, but the intensity appeared to be no more than 3+, even at the 1:5 dilution. Although fluorescent foci of infected cells could be observed with the influenza type B conjugate, the presence of numerous fluorescent particles made reading very difficult. Cross reactivity between influenza type B infected cells and influenza type A conjugates was also observed.

The viral conjugates purchased from Microbiological Associates were not satisfactory, due to low antibody titers and the presence of significant background fluorescence.

SUMMARY

The utilization of the direct immunofluorescence procedure to identify viral isolates will provide rapid and economical alternatives to current methods. The availability of commercially prepared conjugates has significantly enhanced the capability of this laboratory to perform the fluorescent antibody techniques. Studies are now in progress to evaluate the application of the immunofluorescence procedure to clinical specimens.

LITERATURE CITED

1. Hers, J. R.; Kuip, L.; Masurel, N., Rapid Diagnosis of Influenza, *Lancet*, 1:510, 1968.
2. Stevens, T. D. and Watkins, H. M. S., 1969, Rapid Identification of Viruses by Indirect Immunofluorescence: Applied Microbiology, 17:389-393.
3. Smith, C. D. LTC et al, Prophylactic Methods in Prevention of Disease among Army Personnel, Annual Report, August 1972.
4. Liu, C., 1969, Fluorescent Antibody Techniques, p. 179-204. Lennette, E. H. and Schmidt, N. J. (Ed.), Diagnostic Procedures for Viral and Rickettsial Disease, 4th Ed. American Public Health Assoc., Inc., New York.
5. D'Alessio, D; Williams, S.; Dick, E., Rapid Detection and Identification of Respiratory Viruses by Direct Immunofluorescence: Applied Microbiology, August, 1970, p. 233-239, Vol 20, No. 2.

6. Liu, C. 1956, Rapid Diagnosis of Human Influenzal Infection from Nasal Smears by Means of Fluoresceins-labeled Antibody. Proc. Soc. Exp. Biol. Med. 92:883-887.
7. Tatano, I., S. Suzuki, A. Kawamara Jr., H. Kawashima, N. Kusano, Y. Aoyama, A. Sugiura, Y. Akao, K. Oikawa, N. Homma, and M. Naito, 1962, Diagnosis of Influenza by Means of Fluorescent Antibody Techniques, Jap. J. Exp. Med. 32:531-559.
8. Gardner, P. S., McQuillen J., 1968, Letter To The Editor, Lancet 1:597-598.

UPPER RESPIRATORY DISEASE SERUM SURVEY

INTRODUCTION

A survey involving pooled serum samples representative of those likely to be encountered from patients during the FY '77 respiratory disease season were assayed for antibodies by the four Army Reference Laboratories. This survey is performed at least once annually in order to standardize and quality control the complement fixation (CF) procedure as performed by the four Army Reference Laboratories. The CF Procedure is the base line serological screening procedure for detecting diagnostic antibody seroconversions in upper-respiratory-disease patients. For equal participation and unbiased results, the responsibility for preparation and disposition of this survey is rotated to a different Reference Laboratory each year. This year, serum samples were prepared and distributed by the 1st US Army Reference Laboratory.

MATERIALS AND METHODS

Acute and convalescent sera were prepared in pairs to contain predetermined levels of antibodies to Adenovirus, Influenza A, Influenza B, and Mycoplasma, since these are the agents usually encountered during the upper-respiratory-disease season.

Four serum pairs were distributed to each Reference Laboratory (Ft. Baker, Ft. Sam, Ft. Gordon and Ft. Meade). As determined by this laboratory, 4 units of antigen were used for Adenovirus, Flu A, Flu B and Mycoplasma, at dilutions of 1:16, 1:8, 1:16 and 1:4 respectively. Complement in a 1:31 dilution contained 2 units for each antigen except Mycoplasma which contained 2 units in a 1:25 dilution. Sheep red blood cells were standardized at a concentration of 1% by photometry. The standard CF procedure was performed (1).

RESULTS

Table 1 indicates results of complement fixation antibody titrations to Adenovirus, Influenza A, Influenza B, and Mycoplasma. The four Reference Laboratories are identified by the numbers 1, 2, 3 or 4. Since this is not considered an inspection type trouble shooting survey, Reference Laboratories are not identified. As indicated in Table 1, with minor exceptions, the CF titers are in general agreement.

REFERENCE

1. Lennette, E. H., N.A. Schmidt: Diagnostic Procedures for Viral and Rickettsial Infections, 4th Ed. 1969.

TABLE 1. COMPLEMENT FIXATION ANTIBODY TITRATIONS TO THE INDICATED ANTIGENS ON UPPER-RESPIRATORY DISEASE SURVEY SERA

ANTIGEN	LAB ID	SERUM # 101		SERUM # 102		SERUM # 103		SERUM # 104	
		ACUTE	CONVAL	ACUTE	CONVAL	ACUTE	CONVAL	ACUTE	CONVAL
Adenovirus	1	1:8	1:4	1:8	1:16	1:4	1:128	1:4	1:8
	2	1:10	1:5	1:10	1:20	1:10	1:160	1:5	1:10
	3	1:8	<1:8	<1:8	1:16	1:8	1:64	1:8	1:16
	4	<1:8	<1:8	<1:8	1:8	<1:8	1:256	<1:8	1:8
Flu A	1	1:4	≥1:128	1:8	1:32	1:16	1:16	1:16	1:16
	2	<1:5	1:80	1:10	1:40	1:40	1:40	1:20	1:20
	3	<1:8	1:64	1:32	1:64	1:16	1:16	1:16	1:32
	4	<1:8	1:64	1:8	1:64	1:16	1:16	1:16	1:16
Flu B	1	<1:4	<1:4	<1:4	1:32	1:4	1:4	1:4	1:4
	2	<1:5	1:40	1:5	1:40	1:5	1:5	1:10	1:20
	3	<1:8	<1:8	<1:8	1:64	1:8	1:8	1:8	1:16
	4	<1:8	1:32	<1:8	1:32	<1:8	<1:8	1:16	1:16
Mycoplasma	1	<1:4	<1:4	1:4	1:4	1:8	1:8	<1:4	1:64
	2	<1:5	<1:5	<1:5	<1:5	1:5	<1:5	<1:5	1:40
	3	<1:8	<1:8	<1:8	<1:8	<1:8	1:8	<1:8	1:32
	4	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	<1:8	1:64

THE OCCURRENCE OF ENTERO-PATHOGENIC E. COLI IN GASTROENTERITIS INFANTS IN CORRELATION WITH ENTEROTOXINS

INTRODUCTION

Infectious diarrhea is an ubiquitous disease which frequently occurs as epidemic among infants as well as in adult populations.

Fifteen sero groups of E. coli have been implicated in outbreaks of enteritis(1). Identification of strains of E. coli and subsequent serotypings are cumbersome processes revealing little in confirming the organisms role as the causitive agent of clinical illness.

Recently, it has been determined that certain strains of E. coli produce heat-labile and heat stable enterotoxins(2-3). The presence of these enterotoxins and their relationship to gastroenteritis is well documented (4-9).

The three main objectives of this study are to (1) evaluate the mouse Y-1 adrenal cell assay for detecting E. coli heat labile enterotoxin, (2) determine the relationship between enterotoxin production and a particular E. coli serotype, and (3) to determine the relationship between E. coli enterotoxin production and acute gastroenteritis in infants.

MATERIALS AND METHODS

Rectal swabs were collected from infants seen at the Pediatric Clinic, LAMC. These swabs were taken from children presenting with acute diarrhea and also from age-matched children attending the well-baby clinic. Swabs were planted in enteric isolation media (5% Blood agar, MacConkey agar, HEK, GN). Several suspected E. coli (at least five) subsequently grown were picked and biochemically and serologically tested as described by Ewing(10). Lee serotyping reagents were utilized for all serotyping procedures. Toxicity studies were carried out using the Mouse Y-1 adrenal cell assay (6). Y-1 adrenal cells were purchased from the American Type Culture Collection and grown on Ham's F-10 Media supplemented with Horse Serum (15%), Fetal Calf Serum (2.5%), Gentamicin (50mg/ml) and NaHCO₃, ph 7.2. The supernates from centrifuged broth cultures of E. coli were added to cultures of Y-1 adrenal cells which are extremely sensitive to the heat-labile toxin and demonstrably round up in its presence. Known positive and negative controls were included with each run. A blind toxogenic E. coli culture was also included as a control measure with each run. The only major modification to the described procedure was the use of 24 hole plates (Falcon #3008) instead of the standard 96 hole microtiter plates.

RESULTS

The data comparing entero-pathogenic E. coli and enterotoxin production is presented in Table I. The procurement of sufficient data concerning the prevalence of toxigenic E. coli in infants was hindered due to the small number of available patients. Only 23 infants were available for the study. Nineteen of the 23 infants were admitted with acute gastroenteritis and four well infants were selected as controls. Enteropathogenic E. coli was cultured from seven sick infants and two of the control infants. A total of 102 colonies from the sick infants were picked for serotyping of which 14 were enteropathogenic. Seventeen E. coli colonies from the well infants were picked for serotyping of which two were enteropathogenic. As indicated in Table I, none of the 16 enteropathogenic E. coli strains from sick or well infants were toxigenic.

In order to provide sufficient data for the evaluation of the Y-1 adrenal cell assay, clinical E. coli isolates received for serotyping and non-clinical E. coli isolates (water survey, stock culture, other miscellaneous sources) were tested for pathogenicity and production of heat-labile enterotoxin. A total of 235 E. coli colonies were assayed for enterotoxin. As indicated in Table I, none were found to produce enterotoxin, even though 63 isolates were serotyped to be enteropathogenic.

Previously proven isolates including 84 toxigenic and 95 non-toxigenic E. coli cultures were tested as known controls and confirmed. Twenty blind toxigenic E. coli were tested with 100% correlation of enterotoxin production.

Three cultures assayed for the heat-labile toxin produced ambiguous results. These cultures along with our enteropathogenic E. coli toxin producers were sent to CDC for confirmation. The three cultures were reported as negative and our controls as positive by CDC.

SUMMARY

In order to correlate the appearance of heat-labile enterotoxin and acute gastroenteritis in infants, further studies are required involving a larger number of infants. However, significant data was generated in evaluating the Mouse Y-1A cell assay procedure. A total of 354 E. coli isolates were studied of which 79 were serotyped to be enteropathogenic. None of the E. coli were found to produce the heat-labile enterotoxin. The results of the known controls, the blind positive controls, and confirmation of those specimens submitted to CDC indicate that the Mouse Y-1A assay is a sensitive and practical test for the detection of E. coli heat-labile enterotoxin. The Mouse Y-1A assay is currently in use by this laboratory for detecting the enterotoxin.

TABLE 1. A COMPARISON OF ENTEROPATHOGENIC E. COLI
AND ENTEROTOXIN PRODUCTION

<u>E. coli Source</u>	# of E. coli Isolates	# of Entero- pathogenic E.coli Serotypes	# of Patients Positive for E. Coli Pathogens	Enterotoxin Demonstrated
Gastroenteritis Infants	102	14	7/19	0
Well Infants	17	2	2/4	0
Other Clinical <u>E. Coli</u> Isolates	137	37	H/A	0
Non-Clinical <u>E. Coli</u> Isolates	98	26	H/A	0
TOTAL	354	79	9/23	0

LITERATURE CITED

1. Neter, E: Enteropathogenicity of Escherichia coli. Am. J. Dis. Child 129:666-667, 1975.
2. Jacks, T.M., Wu, B.J.L Biochemical Properties of Escherichia coli Low-Molecular Weight, Heat-Stable Enterotoxin. Infant Immun. 9:342-347, 1974.
3. Kantor, H.S., Tao, P., Sorback, S.L: Stimulation of Intestinal Adenyl Cyclase by Escherichia coli Enterotoxin: Comparison of Strains from an Infant and an Adult with Diarrhea. J. Infant Dis. 129:1-9, 1974.
4. Gyles, C.L: Relationships among Heat-Labile Enterotoxins of Escherichia coli and Vibrio cholerae. J. Infant Dis. 129:277-283, 1974.
5. Sack, R. B., Jacobs, R., Mitra, R: Antitoxin Responses to Infections with Enterotoxigenic Escherichia coli. J. Infect. Dis. 129:330-335, 1975.
6. Donta, S.T., Moon, H.W., Whipp, S.C.: Detection of Heat-Labile Escherichia coli Enterotoxin with the Use of Adrenal Cells in Tissue Culture. Science 183:334-336, 1974.
7. Sack, D.A., Merson, M.H., Wells, J. G., Sack, R. B., Morris, T.K.: Diarrhea Associated with Heat-Stable Enterotoxin-Producing Strains of Escherichia coli. Lancet 7928:239-241, 1975.
8. Du Pont, H. L., Formal, S. B., Hornick, R. B., et al: Pathogenesis of Escherichia coli Diarrhea. N. Engl. J. Med. 285:1-9, 1971.
9. Sack, R. B., Gorbach, S. L, Banwell, J. G., et al: Enterotoxigenic Escherichia coli Isolated from Patients with Severe Cholera-like Disease. J. Infant Dis. 123:378-385, 1971.
10. Ewing, William H.: Differentiation of Enterobacteriaceae by Biochemical Reactions - Revised. DHEW Publication No. (CDC) 74-8270, August 1973.

PENICILLINASE-PRODUCING NEISSERIA GONORRHOEAE SURVEILLANCE

INTRODUCTION

In early 1976 the first case of penicillinase-producing Neisseria gonorrhoeae (PPNG) was reported in Maryland in a member of the U.S. Air Force whose last duty assignment was in the Philippines(1). Since that time, numerous cases have been reported in other returnees from the Far East area and their primary contacts. In this study, all Neisseria gonorrhoeae (GC) isolates were tested for sensitivity to penicillin, and those found resistant were further assayed for beta-lactimase production.

MATERIALS & METHODS

Two major groups of personnel were surveyed for PPNG: 1. Those military personnel undergoing separation physicals whose last tour of duty was in Japan, Korea, Philippines, Hawaii, or other Pacific stations and who either exhibited gonorrhoeal symptoms or expressed fear of contact, and 2. San Francisco Bay Area personnel who demonstrated failure of cure with penicillin therapy after 3-7 days. Permanent change of duty personnel were cultured on Transgrow medium at the Oakland Army Base Clinic and sent to Department of Pathology Reference Laboratory, Letterman Army Medical Center for culture and sensitivity. Bay Area Failure of cure cases were sampled by the Health and Environment Section and forwarded to Department of Pathology Reference Laboratory for culture and sensitivity.

Cultures received in the laboratory were tested for Gram reaction and morphology and, regardless of result a 20% sucrose solution was inoculated for the isolation of possible L-forms. Only Gram negative diplococci were identified and tested for sensitivity to penicillin. Those Neisseria showing resistance (<20mm) to the 10mg penicillin disc were further assayed for the enzyme beta-lactimase (penicillinase).

At the time of original culturing, the patient was interviewed for a personal history, giving such information as sex, race, last duty station, prior treatment for gonorrhea (if any) and, if they were undergoing a separation physical, a forwarding address for follow-up purposes was obtained.

RESULTS AND DISCUSSION

Over an 8 month period, 77 cultures were taken, 14 of which were positive for GC (Summary). Of these 14, 10 were sensitive to penicillin and 4 were resistant. Of the 4 resistant cultures, 3 were ascertained to be negative for beta-lactimase production while 1 was determined to be a PPNG.

All positive GC cultures had been symptomatic and were from male personnel. Eight were from Korea, 1 from Japan, and 4 were from the Bay Area, including the PPNG which had been a treatment failure. There were no L-forms isolated.

SUMMARY

Over an eight month period 77 patients were screened for PPNG, both those arriving from the Far East and local Bay Area treatment failures. One PPNG was isolated and identified, and three other GC cultures proved resistant to penicillin but did not produce beta-lactimase.

SUMMARY OF SURVEY RESULTS OF PPNG STUDY FROM 17 FEBRUARY 1977 TO 12 OCTOBER 1977

SEX: Male - 64
Female - 13

RACE: Caucasian- 27
Negro - 36
Oriental - 1
Other - 13

Last or Current Duty Station: Japan - 1
Korea - 42
Hawaii - 2
Bay Area - 20
Other - 12

Culture Results: Negative - 63
Positive - 14 (all male)

Penicillin Sensitivity: Sensitive - 10
Resistant - 4

Resistant Culture Assay for Beta-Lactimase: Positive - 1
Negative - 3

Number Positive L-Form Isolates - 0

Total Cultures Tested: 77

REFERENCES

1. Culliton, B. J. Science, 194:1395-1397, 1976.